Resistance Gene Analogs within an Introgressed Chromosomal Segment Derived from *Triticum ventricosum* That Confers Resistance to Nematode and Rust Pathogens in Wheat

S. Seah,^{1,2} W. Spielmeyer,¹ J. Jahier,⁴ K. Sivasithamparam,³ and E. S. Lagudah¹

¹CSIRO Plant Industry, GPO Box 1600, Canberra A.C.T. 2601, Australia; ²Plant Sciences, ³Soil Science and Plant Nutrition, University of Western Australia, Nedlands W.A. 6907, Australia; ⁴INRA, Station d'Amelioration des Plantes, BP 2935650 Le Rheu, France
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A resistance (R) gene-rich 2S chromosomal segment from *Triticum ventricosum* contains a cereal cyst nematode (CCN; *Heterodera avenae*) R gene locus *CreX* and a closely linked group of genes (*Sr38*, *Yr17*, and *Lr37*) that confer resistance to stem rust (*Puccinia graminis* f. sp. *tritici*), stripe rust (*P. striiformis* f. sp. *tritici*), and leaf rust (*P. recondita* f. sp. *tritici*) when introgressed into wheat. The 2S chromosomal segment from *T. ventricosum* is further delineated in translocations onto chromosome 2A of bread wheat, where the rust genes are retained but not the *CreX* gene. Using these critical genetic stocks, we have isolated family members of R gene analogs that are associated with either the 2S segment from *T. ventricosum* carrying the *CreX* locus or the rust genes. Derivatives of the *Cre3* candidate R gene sequence and a rice (*Oryza sativa*) R gene analog that mapped to the 2S homologous chromosome groups in wheat were used to isolate related gene sequences from *T. ventricosum* that contain a nucleotide binding site-leucine rich repeat domain. The potential of these gene sequences as entry points for isolating candidate genes or gene family members of the *CreX* or rust genes and their further applications to plant breeding are discussed.

Additional keywords: genomic library, linked rust genes, wheat genetic stocks.

The wild wheat species, *Triticum ventricosum* Ces. (syn. *Aegilops ventricosa* Tausch.), is an allotetraploid with the genome designation D'D'N'N' derived from the hybridization of the "D" genome from *A. tauschii* (Coss.) Schmal (syn. *T. tauschii*.) and the N genome from *T. uniariatum* (Vis.) Richter (Kimber et al. 1983; Kimber and Zhao 1983). *T. ventricosum* is the source of several disease resistance (R) genes that are of agronomic importance and have been successfully

introgressed into wheat. These genes include *Sr38*, *Yr17*, and *Lr37*, which are present in "VPM"-derived wheats as a closely linked group that confers resistance to stem rust (*Puccinia graminis* f. sp. *tritici*), stripe rust (*P. striiformis* f. sp. *tritici*), and leaf rust (*P. recondita* f. sp. *tritici*) (Bariana and McIntosh 1993, 1994), respectively. Independently of the "VPM" source, genes conferring resistance to the cereal cyst nematode (CCN; *Heterodera avenae* Woll.), *CreX*, and a leaf rust R gene designated *Lr* have been localized onto a 2S chromosomal segment from *T. ventricosum* (Bonhomme et al. 1995; Jahier et al. 1996). The *Lr* gene has since been shown to express *Lr37* specificity (H. Bariana, *personal communication*) and to be tightly linked to the *Yr17* gene (Robert et al. 1999). It is therefore likely that *Lr* and *Lr37* are identical genes located within the linked group of R genes including *Yr17* and *Sr38* (Bariana and McIntosh 1993, 1994) and present on the 2S segment of *T. ventricosum*. This group of rust genes can be separated from the *CreX* gene in wheat lines containing the introgressed 2S segment that carry the rust genes (present in the genetic stock, L22) but not the *CreX* gene (see Figure 1; Bonhomme et al. 1995; Jahier et al. 1996).

The aim of this study was to isolate and characterize specific R gene family members that are associated with either the *CreX* or the rust genes (*Sr38*, *Yr17*, and *Lr37*) on the 2S chromosomal segment from *T. ventricosum*. An R gene analog (RGA) from barley (*Hordeum vulgare*) (Seah et al. 1998) that was closely related to the CCN *Cre3* candidate R gene sequence from the D genome of wheat (Lagudah et al. 1997) and a rice (*Oryza sativa*) RGA that mapped to the 2S homologous region in bread wheat (Spielmeyer et al. 1998) were used to isolate related candidate R gene sequences from a *T. ventricosum* genomic library. In this study, we show that these newly isolated RGAs that contained a nucleotide binding site-leucine rich repeat (NBS-LRR) domain were derived from the 2S chromosomal segment of *T. ventricosum* that had been introgressed into wheat. We demonstrate that these RGAs are present within specific regions of the 2S segment from *T. ventricosum* that contained either the *CreX* gene or the group of rust genes (*Sr38*, *Yr17*, and *Lr37*). Characterization of RGAs and their family members from the 2S segment of *T. ventricosum* will provide the basis for developing tightly

Corresponding author: E. S. Lagudah
E-mail: e.lagudah@pican.pi.csiro.au

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linked molecular markers and isolating candidate genes for these important R genes in wheat.

RESULTS

Identification of R gene sequences from the 2S segment containing the *CreX* gene.

The ssCH4 RGA, a derivative of the *Cre3* candidate R gene sequence (Seah et al. 1998) identified up to 10 gene family members in *SacI*-digested genomic DNA of *T. ventricosum* accessions no. 10 (not shown) and no. 11 (Fig. 2). Out of these 10 hybridizing bands, three gene members (labeled 1, 2, and 4) that can also be assayed by restriction fragment length polymorphisms (RFLPs) generated from *HindIII* were initially localized to the 2S chromosomal segment of 6N^v (Fig. 1). Chromosome 6N^v in wheat is present as translocated segments of group 2S and 6N^v chromosomes derived from *T. ventricosum* (Bonhomme et al. 1995). These three gene members de-

Fig. 1. Diagrammatic illustration of chromosomes in wheat (*Triticum aestivum* cv. Moisson) genetic stocks with the introgressed group 2 short arm (2S) segment from *T. ventricosum* used to localize the *CreX* and *Sr38*, *Yr17*, and *Lr37* rust genes. Cytological evidence suggests that chromosome 6N^v (derived from *T. ventricosum*) present in the wheat cv. Moisson consists of a 2S segment translocated to the long arm of chromosome 6N^v. **A**, Chromosome containing the 2S introgressed segment present in the Moisson wheat background as a 6N^v(6D) chromosome substitution line (6N^v(6D)) or 6N^v chromosome addition line (M+6N^v). These two wheat lines are resistant to cereal cyst nematode and stem, stripe, and leaf rust. **B**, Chromosome present in the Moisson wheat background as a 6N^v deleted chromosome addition line (M+6N^v del) that does not contain the 2S introgressed segment. These wheat lines are susceptible to cereal cyst nematode and stem, stripe, and leaf rust. **C**, Chromosome containing part of the 2S introgressed segment present in the Moisson wheat background as a 2S-2AS.2AL wheat translocation line (MX12 and MX22). These two lines are resistant to stem, stripe, and leaf rust but have lost resistance to the cereal cyst nematode. Unshaded region depicts the 2S homoeologous segment from *T. ventricosum*; shaded region depicts the 6N^v chromosome in wheat; hatched region depicts the wheat chromosome 2A. (Based on information from Bariana and McIntosh 1993; Bonhomme et al. 1995; Jahier et al. 1996.)

rived from *T. ventricosum* were only present in the Moisson+6N^v chromosome addition line and 6N^v(6D) substitution line but absent in the Moisson (Moi) recurrent parent and the Moisson+6N^v (del) deleted addition line (Figs. 1 and 2). These gene members were absent in the derivatives of the 6N^v lines (MX12 and MX22 wheat translocation lines derived from L22), which have lost the *CreX* gene but retained the rust genes (Figs. 1 and 2). Thus, the 2S segment from *T. ventricosum* carrying the *CreX* gene and three *Cre3*-related gene members can be separated from the rust genes located elsewhere on the 2S chromosomal segment.

Sequence characterization of RGAs associated with the *CreX* segment.

Eleven distinct lambda clones containing recombinant DNA between 15 and 20 kb in length were isolated from a *T. ventricosum* accession no. 11 genomic library screened with the ssCH4 RGA. Two of the isolated lambda clones, designated V2 and V5, when digested with the *SacI* restriction enzyme (or *HindIII*) yielded ssCH4-hybridizing RFLP fragments of sizes identical to those of gene members 1 and 2, respectively (Fig. 2). A subclone derived from a third ssCH4-hybridizing lambda clone, designated V6, when used as a probe, was also found to hybridize only to the wheat lines (Fig. 1) carrying the 2S segment of *T. ventricosum* (not shown). The V6 clone contained an RFLP fragment corresponding not only to gene member 6 in *T. ventricosum* (Fig. 2) but also to a band of similar size present in the Moisson wheat background (Fig. 2).

Fig. 2. DNA hybridization pattern of the barley ssCH4 RGA (spanning the nucleotide-binding site region) on *SacI*-digested genomic DNA of wheat cv. Moisson (Moi), M+6N^v chromosome addition line, M+6N^v (del) deleted chromosome addition line, 6N^v(6D) chromosome substitution line, MX12 and MX22 wheat translocation lines, E30.82 (R)/E30.57 (S) sister lines, Trident (R)/Spear (S) wheat near-isogenic lines (NILs) and *Triticum ventricosum* accession no. 11 (*T. vent. 11*). Arrows pointing toward restriction fragment length polymorphism (RFLP) fragments from *T. ventricosum* (labeled 1–10) hybridizing to the ssCH4 RGA are those potentially associated with the *CreX* gene locus on the 2S segment. Fragment 6 depicted with a broken arrow is masked in certain wheat backgrounds such as Moisson; however, it has been shown to be associated with the segment carrying *CreX* but not the rust genes. Letters in parentheses following wheat NILs indicate resistance (R) or susceptibility (S) to the rust *Sr38*, *Yr17*, and *Lr37* specificities.

In other wheat backgrounds that carried the rust genes but not *CreX*, the RFLP from the V6-specific subclone was absent.

Partial DNA sequences spanning the NBS domain were obtained from clones V2 and V6 (Fig. 3). The V6 amino acid sequence was 89% similar and 83% identical to that of the *Cre3* candidate gene amino acid sequence, compared with 70% similarity and 51% identity between V2 and *Cre3* candidate amino acid sequences. The V2 and V6 amino acid sequences were found to be 72% similar and 52% identical to one another within the NBS region. Comparison of sequences flanking the NBS domains of the V2 and V6 R gene sequences showed no homology to the *Cre3*-derived gene sequence or any sequences present in the GenBank data base as determined by a BLAST search. It was therefore likely that these may constitute intron sequences flanking the NBS domains of these two R gene sequences.

Other NBS-LRR gene members are present on the 2S segment carrying the rust gene cluster.

The screening of the *T. ventricosum* accession no. 11 genomic library with the rice RGA (pic 22) that previously mapped to the short arm of group 2 chromosomes in wheat (Spielmeyer et al. 1998) yielded one distinct clone, designated Vrgal. A complex hybridization pattern was observed in *T. ventricosum* and the other wheat genetic stocks when the csVrgal1-NBS subclone containing the NBS domain encoded

within the Vrgal gene sequence was used as a probe (Fig. 4). A family of five Vrgal gene sequences was present in both *T. ventricosum* accession nos. 10 and 11 (Fig. 4), although some of the gene members could be distinguished by their unique RFLP. The five hybridizing bands from *T. ventricosum* accession no. 10 were also present in the Moisson wheat lines containing the 6N^v chromosome addition and 6N^v(6D) chromosome substitution; both lines carry the 2S segment of *T. ventricosum*, but these bands were absent in the Moisson wheat and M+6N^v (del) lines that do not contain the 2S segment (Fig. 4). These diagnostic bands identified in *T. ventricosum* accession no. 10 were also present on the rust resistant translocation lines (MX12 and MX22) that lacked the *CreX* gene (Fig. 4). Additionally, it was found that the triple rust resistant wheat lines of VPM origin, e.g., Trident and line E30.82, contained the diagnostic bands while the corresponding susceptible wheat isolines did not (e.g. Spear, line E30.57; Fig. 4). These results indicate that the Vrgal gene sequence belongs to a family of five gene members that were co-localized on the 2S chromosomal segment with the rust genes *Sr38*, *Yr17*, and *Lr37* present in *T. ventricosum* accession no. 10 and distinct from the chromosomal segment containing the *CreX* gene.

T. ventricosum accession no. 11 also contained five Vrgal hybridizing fragments of which only two (B and D) corresponded in size to the gene members from *T. ventricosum* ac-

Fig. 3. Amino acid sequence alignment within the nucleotide-binding site (NBS) region of the cereal cyst nematode *Cre3* candidate resistance (R) gene sequence (Lagudah et al. 1997) and the V2 and V6 gene sequences derived from the group 2 short arm (2S) segment of *Triticum ventricosum* carrying the *CreX* gene. Conserved motifs present within the NBS domain of these R gene sequences: I: p-loop; II: kinase-2; III: kinase-3; IV: 'GLPL(A/L)A'; V: 'RCFAYCS'; VI: 'EGF'; and VII: 'HDL'. Flanking sequences beyond the p-loop (I) and 'HDL' (VII) conserved amino acid motifs show no homology to one another.

cession no. 10 that had been introgressed into the wheat lines carrying the three known rust specificities on the 2S segment (Fig. 4). This therefore indicates that the Moisson wheat lines containing the 2S translocated segment carrying the *CreX* gene and the rust genes (Fig. 1) were more likely to be derived from *T. ventricosum* accession no. 10 than from accession no. 11, confirming previous observations by Bonhomme et al. (1995).

Sequence characterization of the *Vrga1D* gene family members.

The *Vrga1* lambda clone as described previously was isolated from a *T. ventricosum* accession no. 11 genomic library

with a rice RGA probe (pic 22) that was specific to the group 2 homologous chromosomes in wheat (Spielmeyer et al. 1998). A *Dra*I digest of the *Vrga1* lambda clone released a hybridizing DNA fragment corresponding in size (not shown) to the *Vrga1D* gene member that was present in both accessions of *T. ventricosum* used in this study (Fig. 4). Sequencing of a 4.9-kb fragment from the *Vrga1* lambda clone revealed the presence of a complete open reading frame resembling that of an NBS-LRR R gene sequence and containing all conserved motifs found within the NBS region of other known NBS-LRR resistance genes (Fig. 5). The *Vrga1D* gene member encoded a putative protein of 1117 amino acids. Because *T. ventricosum* no. 10 was shown to be the likely donor of the introgressed segment into wheat cultivars such as Trident that carry the rust genes *Sr38*, *Lr37*, and *Yr17*, the corresponding *Vrga1* gene member from these two plants was sequenced with oligonucleotide primers from *Vrga1D*. Sequences from *T. ventricosum* no. 10 and Trident were 100% identical at the nucleotide level and the deduced amino acid sequences revealed eight amino acid changes relative to *Vrga1D* in the LRR region (Fig. 5).

Among numerous R gene sequences identified by a BLAST data base search, the *I2C-2* (Ori et al. 1997) R gene homolog from the *I2* gene locus in tomato that confers resistance to Fusarium wilt (Simons et al. 1998) showed the highest similarity to *Vrga1D* across the entire NBS-LRR region. Comparison of the *Vrga1D* gene amino acid sequence spanning the NBS domain with the V2 and V6 R gene sequences and the *Cre3* candidate gene sequence (Lagudah et al. 1997) showed sequence similarities/identities of 54/30, 58/35, and 58%/33%, respectively. The divergence between the latter group of sequences and *Vrga1D* was further demonstrated by the absence of cross-hybridization between their respective probes. This observation suggests that the *Vrga1D* and the *Cre3*-related gene sequences (V2, V6) belong to different gene family members within the NBS-LRR superfamily.

A subclone of the *Vrga1* lambda clone designated cs*Vrga1*-3', derived from the 3' noncoding region of the *Vrga1D* gene sequence, was also shown to detect the introgressed segment that correlates with the presence of the rust genes *Sr38*, *Lr17*, and *Lr37* in wheat (Fig. 6). Two RFLP fragments (approximately 0.4 and 1.5 kb in size) assayed by cs*Vrga1*-3' subclone were diagnostic for the presence of the rust genes *Sr38*, *Yr17*, and *Lr37* in the two sets of wheat near-isogenic lines (NILs) used in this study (Fig. 6). Presence of the diagnostic bands was also consistent with the presence of the rust genes from *T. ventricosum* in an additional 12 sets of independently derived wheat NILs carrying this cluster of rust genes from *T. ventricosum* (Seah 1999).

The cs*Vrga1*-3' clone also detected another RFLP (approximately 0.2 kb in size) that behaved as an allelic marker relative to the bands associated with the rust genes (Fig. 6). Moisson wheat (+6N^v) chromosome addition line and 6N^v(6D) chromosome substitution line carried both the putative allelic bands (Fig. 6). In these two lines, the 2S segment of *T. ventricosum* was translocated onto chromosome 6N^v. In the other genetic lines that carry either an unaltered 2AS chromosomal segment or the homologous segment of 2AS that had recombined with the 2S segment carrying the rust gene cluster from *T. ventricosum*, only one form of the allelic variant remained (Fig. 6). Such allelism confirms the homol-

Fig. 4. DNA hybridization patterns of the cs*Vrga1*-NBS (nucleotide-binding site) subclone (derived from the NBS region of the *Vrga1D* gene sequence) on *Dra*I-digested genomic DNA from *Triticum ventricosum* accession nos. 10 and 11 (*T. vent.* 10 or 11), wheat cv. Moisson (Moi), M+6N^v chromosome addition line, M+6N^v (del) deleted chromosome addition line, 6N^v(6D) chromosome substitution line, MX12 and MX22 wheat translocation lines, E30.82 (R)/E30.57 (S) sister lines, and Trident (R)/Spear (S) wheat near-isogenic lines (NILs). Hybridizing bands labeled A–E are gene family members of the *Vrga1D* gene sequence. *Vrga1* gene members shown with arrows were co-localized with the rust resistance genes (*Sr38*, *Yr17*, and *Lr37*) on the group 2 short arm (2S) segment from *T. ventricosum* no. 10 introgressed into wheat. Letters in parentheses following wheat NILs indicate resistance (R) or susceptibility (S) to the rust *Sr38*, *Yr17*, and *Lr37* specificities.

ogy between the *T. ventricosum* 2S chromosomal segment with chromosome 2AS of hexaploid wheat.

DISCUSSION

This study identified and characterized divergent R gene sequences from *T. ventricosum* that contained NBS-LRR domains with previously isolated RGA probes from barley and rice. Critical wheat genetic stocks containing the introgressed 2S chromosomal segment from *T. ventricosum* facilitated the

localization of these gene family members to specific chromosomal regions carrying either the *CreX* locus or the *Sr38*, *Lr37*, and *Yr17* rust genes. (Figs. 2 and 4).

The 2S segment from *T. ventricosum* that carries both the *CreX* and rust genes is flanked by the homologous group 2 RFLP markers *Xpsr 933* and *Xpsr 131*, which are separated by a genetic distance of 48 cM (Devos et al. 1993; Bonhomme et al. 1995) (see Figure 1A). Previous characterization of line L22, from which the translocation lines Mx12 and Mx22 were derived but which lacked the *CreX* locus, delineated the intro-

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1  MDLVLEEALR ALARAVDQFR SLGERQMRRC LLGAGDHVSG KLRE VATQLD
51  QIRGLLWADD DRSSPARMDR LKEALYGIDD LVDDMEYHSL TFQVESSISS
101 KSNRNPLSSA LRLGKR FVSG GGGGGDEASR CRFLKDLDSV ASTLSSLLKQ
151 AQGSGLP PAV PVPDFDASTL LQGGHKVFGR NKELNDIVQM LVEPPSPHCT
201 ACKVVSIVGF GGLGKTTLAQ SVYDDL RVKS HFDLRAWAYV SGKPKDVELA
251 KQILRSANPR YGGSIDKDAT FATLQLKLN R LMSSKRFLIV LDDIWGDDPF
301 TNEAYNEILS PLRSMESGSR IIAVTQTPKV AGMLDASHTY YLNALGADDC
351 WSLIKESALG GWSTHEESTQ ELEQIGRKIA AKLNGLPLAA KLMGGLLGAT
401 KSTKYWRIIS EKEFSGDITL SLLRLSYSYL PGRLKQCFAF CSIFPKNWKF
451 DQTNLVRLWM ANGFIQPQSG TGKRMEDLGT DYFNLLLSRS FFHALRQGRR
501 THYKMMHDLIH DMAVSASTED CCQIEPGMTR RIPSTVRHVS VTTGSLQDVN
551 AAIKILPKNL RTFIVFGNWP HFLEDDSLGK LKNLRALDVC HCDFTTELPPA
601 ISCLFHLRYL SLSRTIRSLP ESISKLLHLQ TLCFEDKCSL DKLPAGISRL
651 VKLRHLGIDM KYIAQLPGIG RLINLQGSVE FRVEKGGGHA LQELKGIGKL
701 HGQLKIKGLD NVFSRDEASK TDMKSKENLR ALTLEWSSAC RFLTPVADCE
751 VLENLQPHKN LKELSIVRYL GVTSPSWLQM ALLRELQSLH LVNCRSLGVL
801 PALGLLP SLE QLHMKE LCTV ERIGHEFYGT GDMAFP SLKV LVLDDFPSLV
851 EWSEVRENPL PCLQRLKIVD CPKLIQVPAF PPSVSELTVE RTLLISNMKL
901 APYSSSRSEI LTLDISTTSV LSRGLFHQRH LASIIVLNIN AGCKHLVAAE
951 GLHTFTSLQK LQLCHSDISD QNLESL LQVL PSLYSFEMID LPNMTSLLVP
1001 ANNSLCTTVT ELQISNCPLL SSVFSLGTFV SLKHLVIEKC PKLTAASFPV
1051 NFWRLTALKV LSISYCTEFQ SLPTCGLPTS IEVLHLVGCH PKLHGNSSNK
1101 TINSQEEVST LHKTLAH

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Fig. 5. Deduced amino acid sequence of the *Vrg1D* gene sequence showing the nucleotide-binding site (NBS) and other conserved motifs (underlined) commonly found in NBS-leucine rich repeat (LRR) disease resistance proteins. The LRR domain spans the regions between amino acid positions 553 and 1086. Amino acids shown in bold refer to the eight substitution differences that distinguish the *Vrg1D* sequence from the corresponding gene member derived from *Triticum ventricosum* no. 10 and Trident wheat (shown below the the *Vrg1D* sequence).

gressed rust genes to a 3.9-cM segment. This segment was flanked by the *Xpsr* 933 and *Xpsr* 150 homologous group 2 markers on the distal region of chromosome 2A (Bonhomme et al. 1995) (see Figure 1C). The physical size representing the genetic interval of 3.9 cM that contains the three rust genes as well as the *Vrg1*-related gene members is currently unknown.

Clustering of homologs or gene members about an R gene locus and the tight linkage between different R gene loci within a small genetic distance has previously been observed (Michelmore and Meyers 1998). Estimates for physical sizes of R gene loci ranges from approximately 35 kb at the *Cf-4/Cf-9* locus in tomato to more than 4 Mb at the *Dm3* locus in lettuce (Parniske et al. 1997; Thomas et al. 1997; Meyers et al. 1998). The corresponding number of gene members within such clusters may range from two at the *Cf-2* locus in tomato (Dixon et al. 1996) to more than 24 at the *Dm3* locus in lettuce (Meyers et al. 1998). A linkage group, separated by not more than 3.8 cM, consisting of three R gene (*Rps2*, *Rmd*, *Rj2*) loci that confer resistance to various pathogen specificities, has also been previously described (Polzin et al. 1994; Kanazin et al. 1996).

No recombination events have as yet been conclusively demonstrated between the three linked rust genes on the 2S

segment in wheat (Bariana and McIntosh 1993). Thus, the following question remains unanswered: Do the *Vrg1D* gene and/or its family members from *T. ventricosum* no. 10 (Fig. 4) represent candidate rust R genes for one or more of the three specificities present on the 2S segment from *T. ventricosum*? Due to the lack of an efficient transformation system for wheat, we are currently pursuing a mutagenesis approach to generate wheat mutants that have lost resistance to the rust specificities conferred by the *Sr38*, *Yr17*, and *Lr37* genes. The generation of mutants may also clarify whether there are three distinct R gene loci within the *T. ventricosum* introgressed segment that confer three rust specificities. Other, unrelated R gene sequences, yet to be identified, may also be present on the critical 2S segment that may confer resistance to the rusts.

It is not clear as yet whether the 2S segment that had been translocated onto chromosome 6N^v was derived from chromosome 2N^s or 2D^s in *T. ventricosum*. The presence of allelic RFLP fragments linked with either the presence or absence of the rust genes (Fig. 6) introgressed into chromosome 2AS (Bariana and McIntosh 1993, 1994) suggests that the 2S segment originated from 2N^s rather than 2D^s. If the translocated 2S segment of *T. ventricosum* were of 2D^s origin, it would have been expected to be preferentially introgressed into 2DS of wheat rather than 2AS.

Some of the loci controlling CCN resistance among wheat and their relatives are likely to belong to the same family of *Cre3*-related sequences. We have shown that other *Cre3*-derived gene sequences when used as probes detect RFLPs associated with other nonhomologous CCN R gene loci (i.e., *Cre1* and *Cre4*) in wheat (Lagudah et al. 1998). In the 2S segment from *T. ventricosum* containing the *CreX* locus, we found two divergent groups of *Cre3*-related gene family members V2 and V6 with 51 to 83% sequence identity at the amino acid level in the region spanning the NBS region (Fig. 3). It is therefore probable that a candidate for *CreX* gene is either a V2 or V6 gene family member.

Polymerase chain reaction (PCR)-derived RGAs have been isolated and characterized from a wide variety of plants and have been associated with specific R gene loci including those for which specificities are unknown (Leister et al. 1996; Kanazin et al. 1996; Collins et al. 1998; Seah et al. 1998; Shen et al. 1998; Spielmeier et al. 1998). Mapping of RGA sequences isolated from the same or other plant species increases the likelihood of identifying candidate R genes or family members that may be associated with disease resistance, as demonstrated in this study. The identification of candidate genes or closely linked gene members is useful because they are tightly linked markers for the introgression and pyramiding of these R genes into breeding stocks through marker assisted selection. The *csVrg1-3'* RFLP marker derived from this study, when converted into a PCR-based marker, may be useful for introgressing this tightly linked group of rust genes on the 2S segment from *T. ventricosum* into wheat.

MATERIALS AND METHODS

Plant material.

Wheat (*Triticum aestivum* L.) genetic stocks described in Figure 1, including the recurrent wheat parent cv. Moisson (Moi) and its derivatives as well as *T. ventricosum* Ces. accession nos. 10 and 11 (*T. vent.*), were employed to localize and

Fig. 6. DNA hybridization patterns of the *csVrg1-3'* subclone (derived from the 3' untranslated region of the *Vrg1D* gene sequence) on *Dra*I-digested genomic DNA from wheat cv. Moisson (Moi), M+6N^v chromosome addition line, M+6N^v (del) deleted chromosome addition line, 6N^v(6D) chromosome substitution line, MX12 and MX22 wheat translocation lines, E30.82 (R)/E30.57 (S) sister lines, Trident (R)/Spear (S) wheat near-isogenic lines (NILs), and *Triticum ventricosum* accession no. 11 (*T. vent. 11*). Letters in parentheses following wheat NILs indicate resistance (R) or susceptibility (S) to the rust *Sr38*, *Yr17*, and *Lr37* specificities. The approximately 0.4 and 1.5 kb restriction fragment length polymorphism (RFLP) fragments are diagnostic for the rust genes on the group 2 short arm (2S) segment whereas the approximately 0.2 kb RFLP fragment identifies the allelic variant in wheat lacking the three rust genes.

associate the isolated R gene sequences with either the CCN (*Heterodera avenae* Woll.) *CreX* gene or the *Sr38* stem rust, *Yr17* stripe rust, and *Lr37* leaf rust genes on the 2S introgressed segment in wheat derived from *T. ventricosum*. Translocation lines Mx12 and MX22 in Moisson wheat background that carry the rust genes but not *CreX* were derivatives of the genetic stock, L22, described by Bonhomme et al. (1995). Two pairs of wheat NILs or sister lines that had been either selfed or backcrossed four to eight generations to the recurrent parent, (1) Trident (R)/Spear (S) and (2) E30.82 (R)/E30.57 (S), were also utilized. The rust genes from *T. ventricosum* present in the resistant (R) wheat cv. Trident were derived from VPM1 wheat (*T. ventricosum*/*T. turgidum* var. *carthlicum* (*T. persicum*)/3**T. aestivum* cv. Marne)). The resistant E30.82 and susceptible (S) E30.57 sister lines were of single-seed descent from the F₇ generation derived from a cross between line B41 and Rendezvous. The Trident/Spear NILs were originally selected for *Yr17* specificity whereas the other pair of NILs was selected for *Sr38* specificity.

All genomic DNA was extracted from leaf material from fo4-ur to 6-week-old plants as described in Lagudah et al. (1991b). Genomic DNA extracted from the genetic stocks (see Figure 1) was digested with *DraI*, *HindIII*, or *SacI* restriction enzymes to analyze for RFLPs. DNA hybridization and washing conditions were as described in Lagudah et al. (1991a).

Isolation and sequence characterization of R gene sequences.

A *T. ventricosum* accession no. 11 genomic lambda library was constructed from 300 µg each of *MboI* or *Sau3AI* partially digested DNA that had been size fractionated on a glycerol gradient. The fractionated and purified *T. ventricosum* DNA was then ligated into LambdaGem-11 *BamHI* arms cloning vector (Promega, Madison, WI) and packaged according to the supplier's instructions.

T. ventricosum accession no. 11 was initially used because it carried *Cre2* genes that are highly effective against CCN (Delibes et al. 1993). The *T. ventricosum* lambda genomic library was screened with the ssCH4 and pic 22 RGAs (Seah et al. 1998; Spielmeyer et al. 1998). The ssCH4 RGA from barley was a derivative of the *Cre3* candidate gene sequence from *A. tauschii* (Lagudah et al. 1997; Seah et al. 1998) that conferred resistance to CCN. The pic 22 RGA from rice (*Oryza sativa*) was previously mapped to the short arm of homologous chromosome group 2 of hexaploid wheat (Spielmeyer et al. 1998) and was kindly supplied by N. Collins (CSIRO Plant Industry, Canberra, A.C.T., Australia). Hybridization and washing conditions of the lambda library filters were as described in Lagudah et al. (1991a).

The purified DNA from each hybridizing genomic lambda clone isolated with the ssCH4 and pic 22 RGA clones was digested with either *SacI*, *BamHI*, or *XhoI* restriction enzymes and subcloned into dephosphorylated pBluescript II phagemid cloning vector (Stratagene, La Jolla, CA). Selected hybridizing subclones were then processed and sequenced according to the supplier's instructions with the ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq DNA polymerase (Perkin Elmer, Melbourne, Australia). The sequences were compared and analyzed with BLAST searches (Altschul et al. 1990) and a suite of programs of the

Genetics Computer Group (GCG) from the University of Wisconsin (Madison).

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